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GENETIC APPROACH(U) ILLINOIS UNIV AT URBANA DEPT OF
MICROBIOLOGY J KONISKY 16 MAY 88 N00014-86-K-0224

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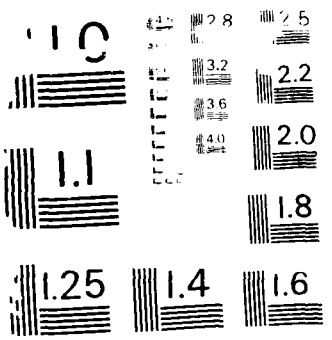

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Characterization of Methanogen Membrane Function: A Genetic Approach

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Santoro, Dharmavaram, Konisky

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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

This research program involves studies on membrane fuction and structure in the methanogenic archaebacterium, Methanococcus voltae. We are particularly interested in the role of membrane-associated ATPase in energetics and the molecular and energetic basis of nutrient active transport.

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DATE: May 11, 1988

PROGRESS REPORT ON CONTRACT N0014-86-K-0224

PRINCIPLE INVESTIGATOR: Professor Jordan Konisky

CONTRACTOR: University of Illinois

CONTRACT TITLE: Methanogen Membrane Function

START DATE: August 1, 1986

RESEARCH OBJECTIVE: To investigate the structure of the cell membrane of the arcahebacterium, Methanoccos voltae, as it relates to mechanisms of energetics; to characterize the structure, function and molecular biology of individual membrane proteins.

PROGRESS (Year 2). We have made substantial progress toward characterizing the Mc. voltae membrane-associated P-type ATPase and have advanced several hypotheses relating its function in overall methanogen energetics. We have been able to demonstrate the presence of a phosphorylated intermediate which provides direct evidence that this enzyme is a P-type ATPase. Significant progress has also been made in purification of the enzyme. With regard to our studies of membrane transport systems, we have developed a sensitive assay for determining the utake characteristics of the methyl-CoM transport system. We have been able to demonstrate that exogenously supplied substrate is quantitatively converted to methane, thus, proving the physiological validity of this transport system. This assay system has allowed us to confirm our previous hypothesis that bromoethanesulfate resistant strains are defective in the methyl-CoM transport system.

WORK PLAN (Year 3): Based on an approved one year no-cost extension, this contract will be carried forward for an additional year. We intend to complete the purification of the ATPase and begin its characterization. We will determine its size, number of subunits and define the function of each subunit. N-terminal regions of the protein will be sequenced and DNA probes prepared which will be utilized in the cloning of the structural gene. As an alternate plan, antibodies willl be raised for use in selection of clones carrying ATPase encoding DNA. We will also attempt to develop a vesicle system which can be used for reconstitution studies. The goal is to determine the nature of the translocated ion. This information is important in our consideration of schemes of energetics. We intend to determine the energetics of methyl-CoM uptake into whole cells and membrane vesicles. These studies will utilize well characterized ionophores as well as artifically generated ion gradients. We intend to begin to identify molecluar components of the methyl-CoM transport system by examining the biochemistry of membranes isolated from cell which are defective in its uptake.

INVENTIONS: None

PUBLICATIONS:

Santoro, N. and J. Konisky (1987). Characterization of bromoethanesulfonate mutants of Methanococcus voltae: Evidence of a coenzyme transport sytem. J. Bacteriol., 169: 660-665.

Dharmavaram, R. and J. Konisky (1987). Identification of a vanadate-sensitive membrane-bound ATPase in the Archaebacterium, Methanococcus voltae. J. Bacteriol., 169: 3921-3925.

TRAINING ACTIVITIES: Two graduate students are currently supported by this ONR contract. One of these students is a citizen of India. In July 1988, a postodoctoral fellow will join the laboratory to work on the ATPase project. He will be supported by this contract.

AWARDS: J. Konisky has been appointed an Associate in the Canadian Institute for Advanced Research.

END

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